FARMING SPONGES FOR THE PRODUCTION OF BIOACTIVE METABOLITES

A.R. DUCKWORTH, C.N. BATTERSHILL, D.R. SCHIEL AND P.R. BERGQUIST

Duckworth, A.R., Battershill, C.N., Schiel, D.R. & Bergquist, P.R. 1999 06 30: Farming sponges for the production of bioactive metabolites. *Memoirs of the Queensland Museum* **44**: 155-159. Brisbane. ISSN 0079-8835.

For successful aquaculture of sponges, with the aim of producing metabolites, a farming method is required that promotes sponge growth and survival, and produces high yields of target metabolites. To help develop a suitable farming method growth and survival were compared for two New Zealand sponges, *Latrunculia brevis* (Ridley & Dendy) and *Polymastia croceus* (Kelly-Borges & Bergquist), experimentally grown in a variety of ways. Explants were farmed in mesh, on rope, and with rope threaded through them. For both species of sponge, survival was greatest for explants farmed in mesh, probably because this produces little tissue damage and prevents explants from dislodging and 'escaping'. This method also promoted highest growth of *L. brevis*, with some explants doubling their weight in two months. The growth of *P. croceus*, however, was highest in explants with rope threaded through them. Explants of both sponges farmed on rope did not attach and had poor growth and survival. These findings are a major step forward in developing a method for farming sponges in temperate waters of New Zealand. ☐ *Porifera, aquaculture, farming method.*

Alan R. Duckworth (email: a.duckworth@niwa.cri.nz), University of Canterbury/NIWA Centre of Excellence in Aquaculture and Marine Ecology, PO Box 14901, Kilbirnie, Wellington, New Zealand; Christopher N. Battershill, National Institute of Water and Atmospheric Research (NIWA), PO Box 14901, Kilbirnie, Wellington, New Zealand; David R. Schiel, Zoology Department, University of Canterbury, Private Bag 4800, Christchurch, New Zealand; Patricia R. Bergquist, Zoology Department, University of Auckland, Auckland, New Zealand; 24 March 1999.

A major obstacle facing sponge aquaculture in the production of metabolites is the lack of a suitable farming method or on-growing structures (Shimizu, 1995; Osinga, 1998). To be suitable for large scale commercial use a structure must be inexpensive, have a low surface area to reduce drag and bio-fouling, and allow cost-effective and efficient harvesting. It must also promote high sponge growth and survival while also maintaining high metabolite production.

Farming structures used to grow bath sponges have historically involved attaching explants to concrete discs, or threading wire through explants so that they hang in mid-water (Cotte, 1908; Moore, 1908; Crawshay, 1939). This last method was modified slightly by Verdenal & Vacelet (1990), who successfully grew commercial bath sponges by first threading plastic-coated metal wires through explants and then attaching them to vertical ropes. Development of new farming structures to grow bath sponges was constrained by market forces determining acceptable shape and size of products (Storr, 1964; Bergquist & Tizard, 1969). In contrast, explant shape has no bearing on efficient metabolite production, and consequently there is considerable flexibility in the development of new farming structures for metabolite aquaculture.

We identified three general farming methods: 1) explants placed in mesh; 2) explants attached to and farmed on rope; 3) explants farmed with thin rope threaded through them (Fig. 1). The first method has already been tested with some success (Duckworth et al., 1997). For each method of farming it was necessary to test variation in structures and materials used. For example, rope thickness and composition were important considerations using methods 2 or 3 - as rope thickness increases, drag pressure as well as capital cost increases accordingly, whereas a decrease in rope thickness produces a decrease in available surface area for explant attachment. Rope composition is also important, because explant growth, survival and metabolite concentration may differ between ropes made of different materials.

In this study, we tested the potential of each farming method using two New Zealand sponges: *Latrunculia brevis* (Ridley & Dendy, 1886), a green massive sponge found throughout New

Zealand waters usually in exposed areas (Battershill & Bergquist, 1999a), and *Polymastia croceus* (Kelly-Borges & Bergquist, 1997), a common orange massive sponge. Both sponges contain metabolites with potential pharmaceutical properties (Lill et al., 1995; National Cancer Institute, personal communication).

The results described here are preliminary and part of a larger, ongoing experiment (October 1998). We focus here on the overall patterns of explant growth and survival between the three farming methods tested. Full results will be published after all relevant experiments are completed.

MATERIALS AND METHODS

For both *L. brevis* and *P. croceus*, we collected approximately forty sponges of similar size at 10-20m depth off the coast of Wellington (41°21S, 174°50E), situated at the southern end of the North Island of New Zealand. These sponges were cut, leaving approximately 30% of the original sponge intact to regenerate. Cut sponges left *in situ* had high survival and quickly healed. All collected sponges were cut under running seawater in a laboratory into cube-shaped explants, approximately 27cm^3 in size and 16g in weight. All explants had at least one side uncut, with the pinacoderm intact.

Three farming methods were tested for each species. Explants were: 1) placed in mesh; 2) attached directly to thick rope; 3) or had thin rope threaded through them (each method has several sub-methods, but full analysis at this stage is not yet possible given that the experiment is still in progress) (Fig. 1). Under method 2, each explant was firmly secured with cotton thread to an individual length of rope measuring 15x2.5cm. All explants in this method had their uncut side (with intact pinacoderm and oscules) facing outwards, away from the rope. Under method 3, to thread thin rope through explants, we carefully pushed a large needle, with rope attached, through each explant. Rope used in this treatment was 2-3mm thick. We used 40 explants of each species for each method. Explants were randomly selected and tied at intervals of 15cm to a rope back-line, and farmed at a depth of 12m.

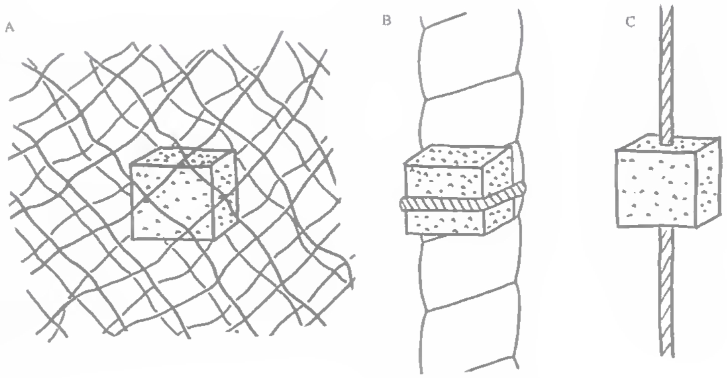

FIG.1. Schematic drawing of the 3 farming methods tested. A, explants placed in mesh; B, explants attached to rope; C, explants with thin rope threaded through them.

We farmed *L. brevis* and *P. croceus* in Wellington Harbour from October 1997 to January 1998, and compared explant growth and survival. Growth was determined by wet-weighing the explants (to 0.1g) at the start and at the end of each experiment. We discovered that explants disturbed 30mins before weighing would expel all excess water, allowing us to weigh their true tissue weight.

Comparisons between the different methods of farming on growth and survival in *L. brevis* and *P. croceus* were made using one-way ANOVA.

RESULTS

In both species growth rates were not significantly different between the three farming methods tested ($F_{df2}=0.24$ and 0.04, N=68 and 110, P>0.05, for *L. brevis* and *P. croceus*, respectively). Conversely, survival of explants was significantly different between the methods used ($F_{df2}=31.28$ and 23.79, N=120, P<0.001, respectively) (Figs 2B,D). Survival of both *L. brevis* and *P. croceus* farmed in mesh, under method 1, was excellent. Only one of the forty explants of *L. brevis* died and all *P. croceus* survived. The growth of *L. brevis* explants farmed in mesh was relatively good with an average weight gain of 1.2g over the 95 days of experimentation (Fig. 2A). Some of these replicates doubled their weight from 16g to over 32g during this period, a promising result given the brief time of experimentation. Many of these explants grew through the mesh, incorporating it into their tissue. In comparison, average growth of *P. croceus* farmed in mesh was poor, increasing only 0.1g in weight over 95 days (Fig. 2C).

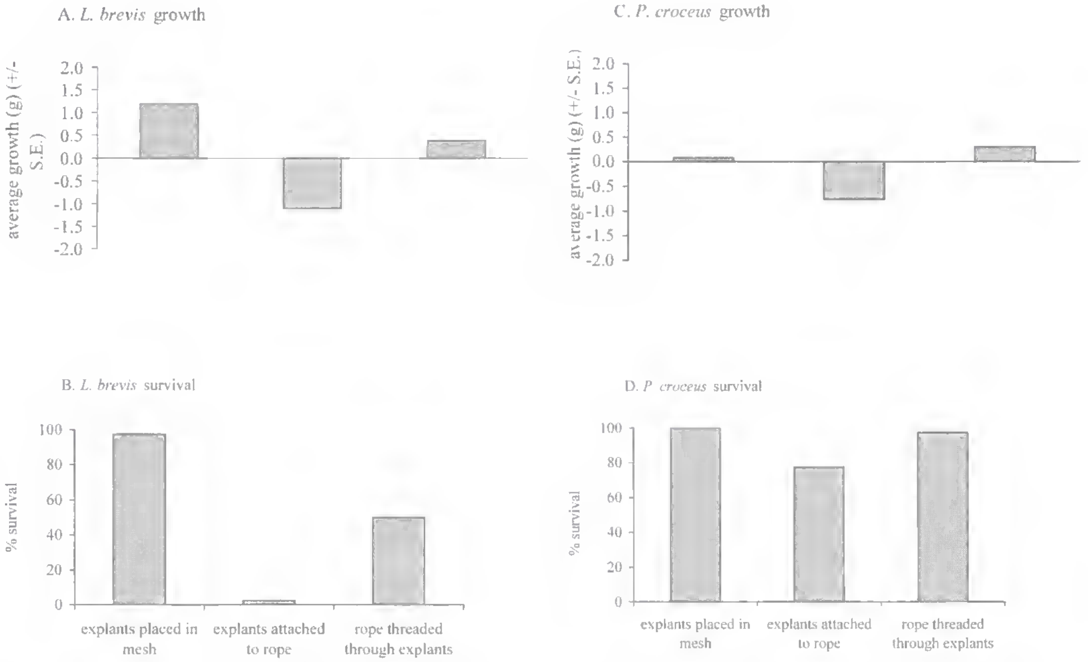

FIG. 2. Comparison in growth and survival of *L. brevis* and *P. croceus* between the three farming methods tested. Growth represents average explant weight gain or loss (+/- S.E.) over 95 days. Survival represents percent survival of the forty explants transplanted in each farming method.

Neither species grew well on rope (method 2). On average, *P. croceus* lost 0.8g while *L. brevis* lost 1.1g over 95 days (Figs 2A,C). Under method 2, survival on rope was also poor. *Polymastia croceus* had 78% survival but only 1 of 40 *L. brevis* explants survived (Figs 2B,D). Under this farming method no explants of either species attached to the rope. The explant side, in contact with the rope, was similar in appearance (morphology and colour) to the other healed sides. We also observed many explants moving or growing away from the rope, ultimately becoming dislodged.

Under method 3, when rope was threaded through explants, all but one *P. croceus* survived the 95 days experiment, whereas only 50% of *L. brevis* survived (Figs 2B,D). Average weight gain for both sponges was similar, approximately 0.3g (Figs 2A,C). Few explants of either species attached to the threaded rope. After 95 days, most explants had changed shape and were moving away from the rope.

DISCUSSION

The importance of choosing a suitable method of farming for sponge aquaculture is well demonstrated in this study. Survival of two species of sponges was greatly affected by the method used. Average growth of both species was generally low for all methods, most probably due to the short (95 day) period of experimentation, and factors inherent to each method mentioned below.

The high survival of *P. croceus* and *L. brevis* farmed in mesh (method 1) may be explained by two factors: 1) Explants experienced the least initial damage, as they are simply placed in mesh. By comparison, explants grown under the other methods had greater disturbance, with rope either pushed through or squeezed around them, causing tissue damage and increased mortality; 2) Even in cases where mesh method is not ideal, explants were effectively trapped in mesh. We noticed many explants in the rope methods moving or growing away from the rope, ultimately becoming dislodged. For farming this is effectively the same as mortality (i.e. the sponge is lost).

One disadvantage of the mesh farming method is a higher rate of fouling of mesh by sediment and sessile organisms, particularly bryozoans, reducing water flow and possibly influencing poor explant growth or even weight loss (Bakus, 1968; Duckworth et al., 1997). Restricted water movement due to fouling probably caused poor growth of *P. croceus* farmed in mesh. Unlike *P. croceus*, many explants of *L. brevis* quickly grew through and over the mesh, reflecting inherent species differences. This reduced the effect of fouling and, combined with low explant stress and damage, probably explains the better growth of *L. brevis* farmed in mesh. Harvesting sponges growing in mesh would involve cutting away tissue growth, leaving the explant behind to grow back through the mesh.

Sponges farmed with rope threaded through them (method 3) were less effected by fouling because they were directly exposed to water. Whereas this may have promoted growth, mortality may have increased because of increased tissue damage. It is likely that increased tissue damage and rejection of the threaded rope caused poor survival of *L. brevis*. In contrast, *P. croceus* farmed with threaded rope survived well. Differences in growth and survival between the two sponges suggest that *P. croceus* is a hardier species and more amenable to different farming methods. However, given a suitable method, *L. brevis* achieved the best combination of growth and survival.

Neither species attached well to the threaded rope, which probably caused reduced growth. Other studies have shown that only explants attached to their fastening wire or identification tag grew well (Verdenal & Vacelet, 1990). The ability of sponges to change shape (Bond & Harris, 1988; Bond, 1992) allows them to move away from unpleasant conditions and can result in loss of explants and low overall survival. This farming method will not succeed unless a rope material is found to which explants will attach. We are currently investigating this, testing explant growth, survival and attachment on threaded rope made of different natural and artificial materials. It is unlikely that this farming method will be suitable in exposed areas where high water movement can easily tear sponges away from the rope.

Many studies have shown that sponges will attach well to a wide variety of natural and artificial substrata (Cotte, 1908; Moore, 1908; Crawshay, 1939; Wulff, 1984, 1985, Barthel & Theede, 1986; Bond & Harris, 1988; Rosell & Uriz, 1992). Unfortunately, both species of sponge in our study failed to attach to any of the ropes tested, perhaps a result of high substrate selectivity shown by some sponges (Battershill & Bergquist, 1999b).

Differences in growth and survival observed in the two species, *L. brevis* and *P. croceus*, probably point to inherent differences in sponge species ability to be successfully farmed. Thus, the findings of this study do not preclude the possibility of farming other New Zealand sponge species on rope. It may be possible to modify this method of farming to improve sponge attachment. For example, Battershill & Bergquist (1999b) discovered that *P. croceus* settles preferentially on rock chips, and it may be possible to incorporated these into the warp of a rope to promote explant attachment. Various types of rope substrate should also be tested.

Many factors have to be considered in the development of a method or on-growing structure suitable for farming sponges for metabolite production. These include cost, bio-fouling, harvesting procedures, explant growth and survival, and metabolite yield. The findings of this study, which concentrated on explant growth and survival using three farming methods, will help develop a suitable on-growing structure for farming massive sponges, such as *P. croceus* and *L. brevis*.

ACKNOWLEDGEMENTS

We would like to thank the many divers that helped in this study, in particular Chris Woods, Pete Notman and Phil James.

LITERATURE CITED

BAKUS, G.J. 1968. Sedimentation and benthic invertebrates of Fanning Island, Central Pacific. Marine Geology 6: 45-51.

BARTHEL, D. & THEEDE, H. 1986. A new method for the culture of marine sponges and its application for experimental studies. Ophelia 25: 75-82.

BATTERSHILL, C.N. & BERGQUIST, P.R. 1990. The influence of storms on asexual reproduction, recruitment, and survival of sponges. Pp. 397-403. In Rüztler, K. (ed.) New perspectives in sponge biology. (Smithsonian Institution Press: Washington D.C.).

1999a. The Porifera. In Cook, S.D.C. (ed.), New Zealand Coastal Invertebrates. Vol. 1. (Canterbury University Press: Christchurch) (in press).

1999b. Novel asexual reproduction in the sponge *Polymastia croceus*: Can sponge buds select settlement sites? Marine Biology. (in press).

BERGQUIST, P.R. & TIZARD, C.A. 1969. Sponge industry. Pp. 665-670. In Firth, F.E. (ed.) The

Encyclopedia of Marine Resources. (Van Nostrand Reinhold Company: New York).

BOND, C. 1992. Continuous cell movements rearrange anatomical structures in intact sponges. Journal of Experimental Zoology 263: 284-302.

BOND, C. & HARRIS, A.K. 1988. Locomotion of sponges and its physical mechanism. Journal of Experimental Zoology 246: 271-284.

CRAWSHAY, L.R. 1939. Studies in the market sponges 1. Growth from the planted cutting. Journal Marine Biological Association of the United Kingdom 23: 553-574.

COTTE, J. 1908. Sponge culture. Bulletin of the Bureau of Fisheries 28: 567-614.

DUCKWORTH, A.R., BATTERSHILL, C.N. & BERGQUIST, P.R. 1997. Influence of explant procedures and environmental factors on culture success of three sponges. Aquaculture 156: 251-267

KELLY-BORGES, M. & BERGQUIST P.R. 1997. Revision of Southwest Pacific Polymastiidae (Porifera: Demospongiae: Hadromerida) with descriptions of new species of *Polymastia* Bowerbank, *Tylexocladus* Topsent, and *Acanthopolymastia* gen. nov. from New Zealand and the Norfolk Ridge, New Caledonia. New Zealand Journal of Marine and Freshwater Research 31: 367-402.

LILL, R.E., MAJOR, D.A., BLUNT, J.W., MUNRO, M.H.G., BATTERSHILL, C.N., MCLEAN, M.G. & BAXTER, R.L. 1995. Studies on the biosynthesis of Discorhabdin B in the New Zealand sponge *Latrunculia* sp. B. Journal of Natural Products 58: 306-311.

MOORE, H.F. 1908. A practical method of sponge culture. Bulletin of the Bureau of Fisheries 28: 545-585.

OSINGA, R., TRAMPER, J. & WIJFFELS, R.H. 1998. Cultivation of marine sponges for metabolite production; process engineering aspects. Trends in Biotechnology 16:130-134.

RIDLEY, S.O. & DENDY, A. 1886. Preliminary report on the Monaxonida collected by H.M.S. 'Challenger'. Part II. Annals and Magazine of Natural History 18: 470-493.

ROSELL, D. & URIZ, M.J. 1992. Do associated zooxanthellae and the nature of the substratum affect survival, attachment and growth of *Cliona viridis* (Porifera: Hadromerida)? An experimental approach. Marine Biology 114: 503-507.

SHIMIZU, Y. 1995. Extracting drugs from the sea. Maritimes University of Rhode Island, Marine Programs 38: 13-16.

STORR, J.F. 1964. Ecology of the Gulf of Mexico sponges and its relation to the fishery. Special Scientific Report Fisheries 466: 1-73.

VERDENAL, B. & VACELET, J. 1990. Sponge culture on vertical ropes in the Northwestern Mediterranean Sea. Pp. 416-453. In Rützler, K. (ed.) New perspectives in sponge biology. (Smithsonian Institution Press: Washington DC).

WULFF, J.L. 1984. Sponge-mediated coral reef growth and rejuvenation. Coral Reefs 3: 157-163.

1985. Dispersal and survival of fragments of coral reef sponges. Proceedings of the 5th International Coral Reef Congress 2: 119-124.